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Poster Presentation

A New Scalable High Resolution Resin for Protein Purification by Hydrophobic Interaction

W. Sawlivich, K. O'Connor, D. Phillips, and George Vella, (Sponsor: J. Li)

Waters Chromatography Division, Milford, MA 01757

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MILLIPORE

Waters Chromatography Division
34 Maple Street
Milford, MA 01757
508 478-2000

INTRODUCTION

Hydrophobic interaction chromatography (HIC) is a technique for separating biomolecules by their degree of hydrophobicity. The exposed non-polar regions on the protein interact with the weakly hydrophobic stationary phase.

In contrast to reversed phase, which is also based on hydrophobic interactions but employs organic solvents, there is little or no denaturation of proteins with HIC. The solute-surface interaction is milder and totally aqueous mobile phases are used with HIC.

The mobile phase consists of a salting-out agent, such as ammonium sulfate, which, at high concentration, retains the protein by increasing the hydrophobic interaction between the solute and the stationary phase. The salt concentration is gradually decreased to elute the protein. Because HIC requires high salt concentrations for binding proteins, it is a natural second step after either ion exchange or salt precipitation.

Retention and selectivity have been shown to be dependent on the pH and type of salt used. The salts which have the stronger "salting-out" ability cause the greater increase in hydrophobic interaction between the protein and the stationary phase. Also, recovery of mass and biological activity in particular salts can be manipulated by varying the salt concentration of the mobile phase.

The most frequently used salt is ammonium sulfate (highly soluble in water and transparent at 280 nm) at concentrations of 2.0 to 1.5 M in a sodium phosphate buffer at pH 7.0.

In order to demonstrate the separation capabilities of the Waters PH-814 material , several protein mixtures were separated and compared to HIC packings offered by other manufacturers.

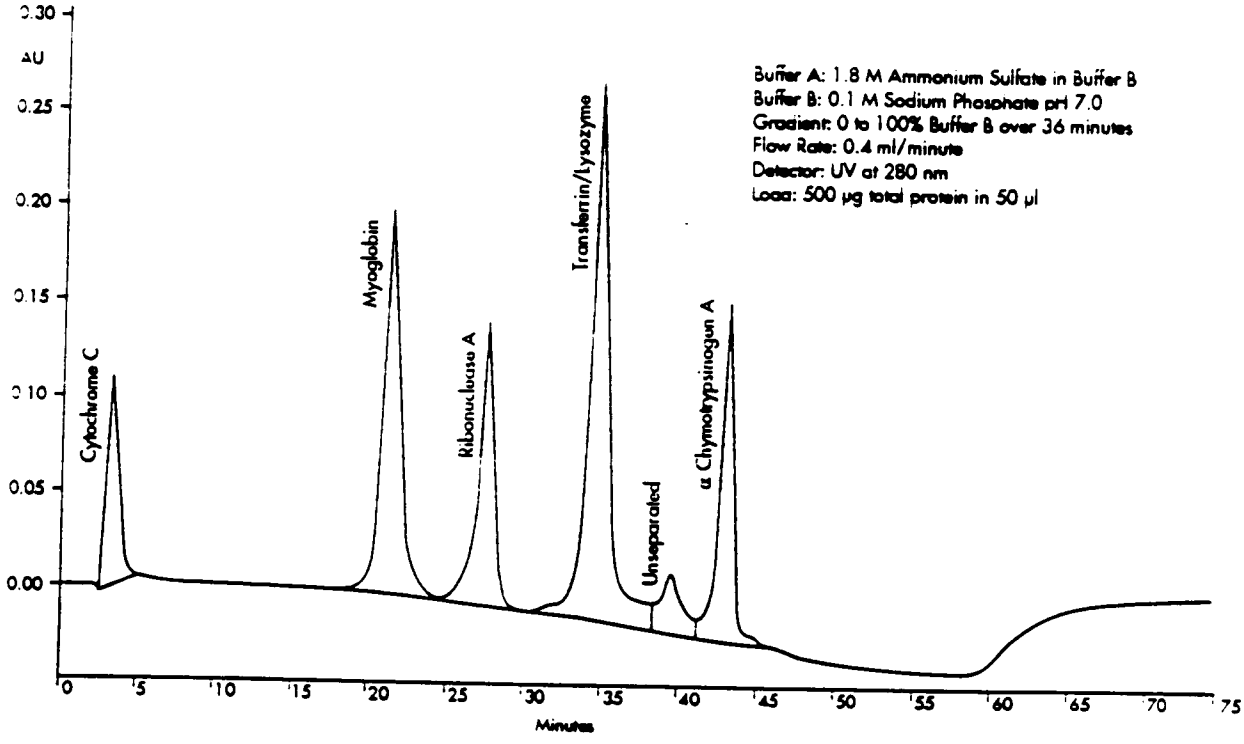
COLUMN DATA

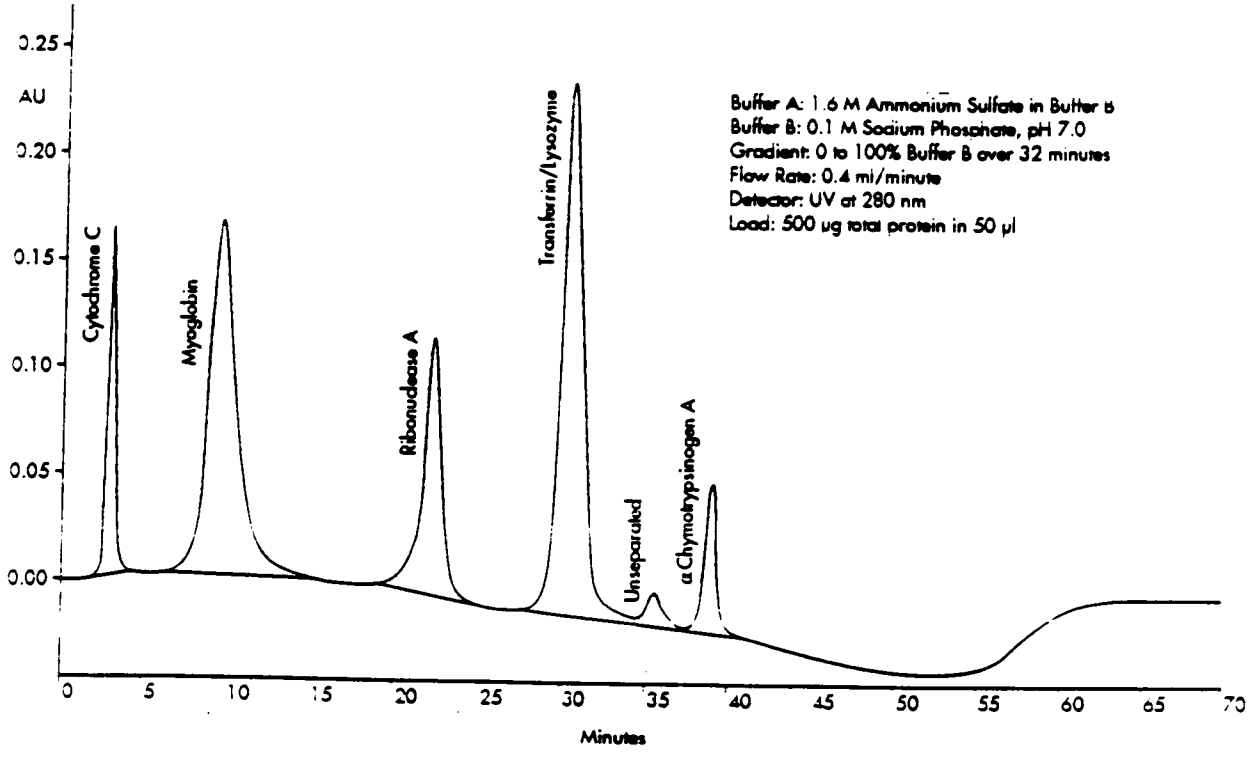
	WATERS PH-814	TSK	PHARMACIA
PARTICLE SIZE	8μm	10μm	13μm
COLUMN	Steel	Glass	Glass
DIMENSIONS	0.8 X 7.5 cm	0.8 X 7.5 cm	0.5 X 5 cm
CAPACITY*	40 mg/ml	25 mg/ml	40 mg/ml

* = CAPACITY OF DYNAMICALLY LOADED OVALBUMIN

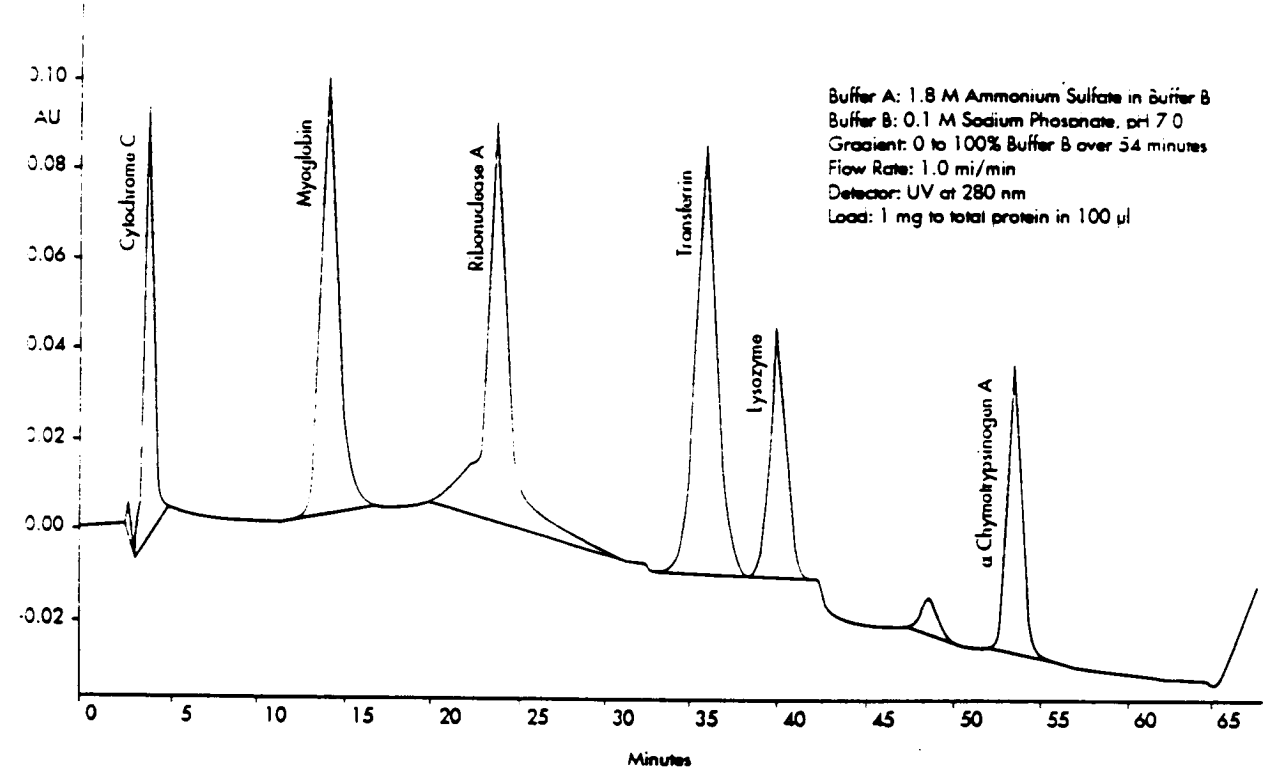
Comparison of Standard Mixtures at 1.8M and 1.6M Ammonium Sulfate in 0.1M Phosphate pH 7.0

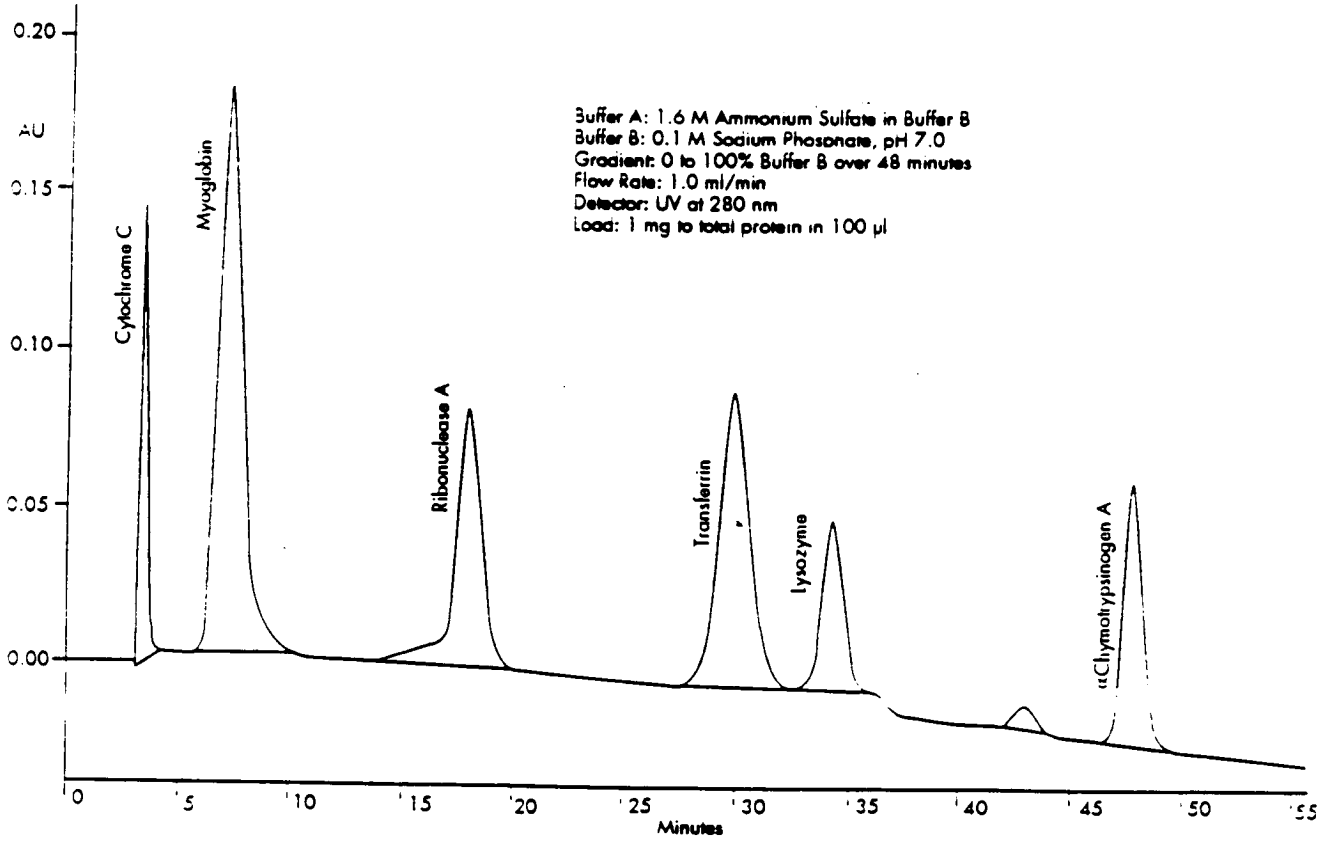
Pharmacia Phenyl Superose (0.5 X 5 cm)



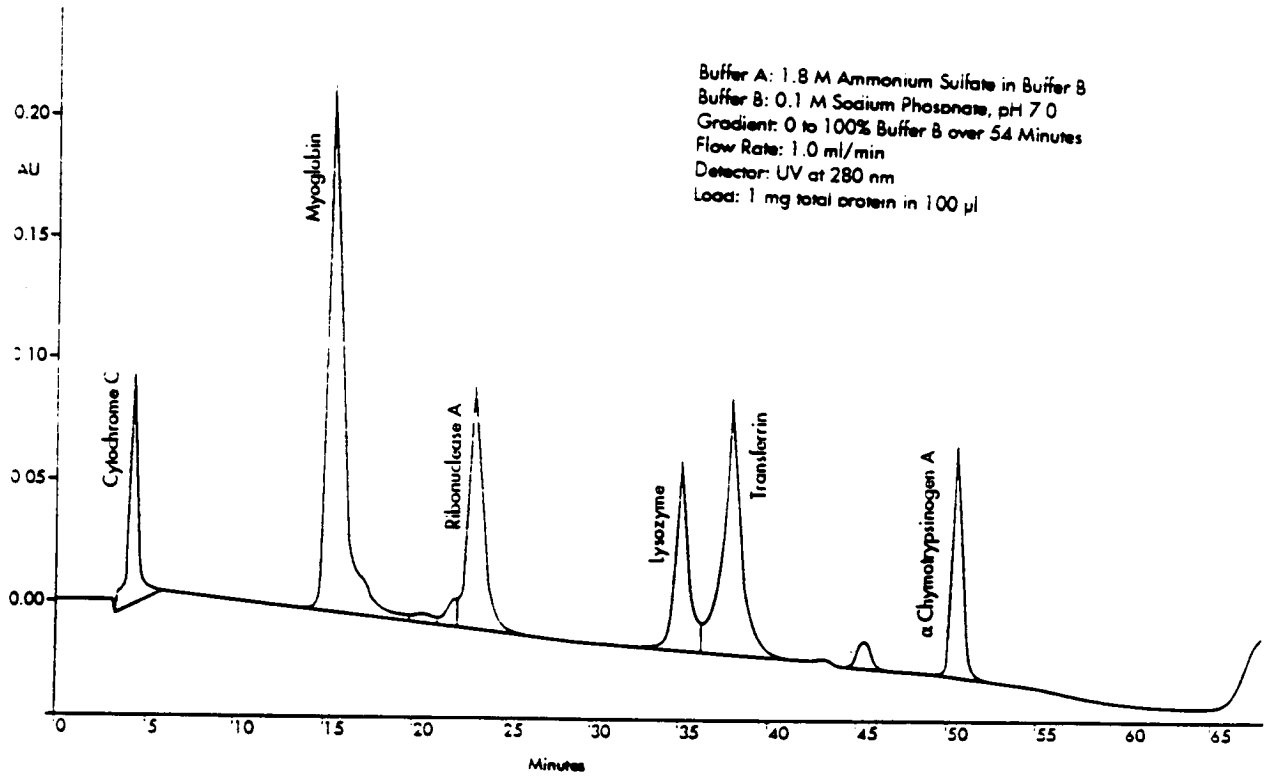


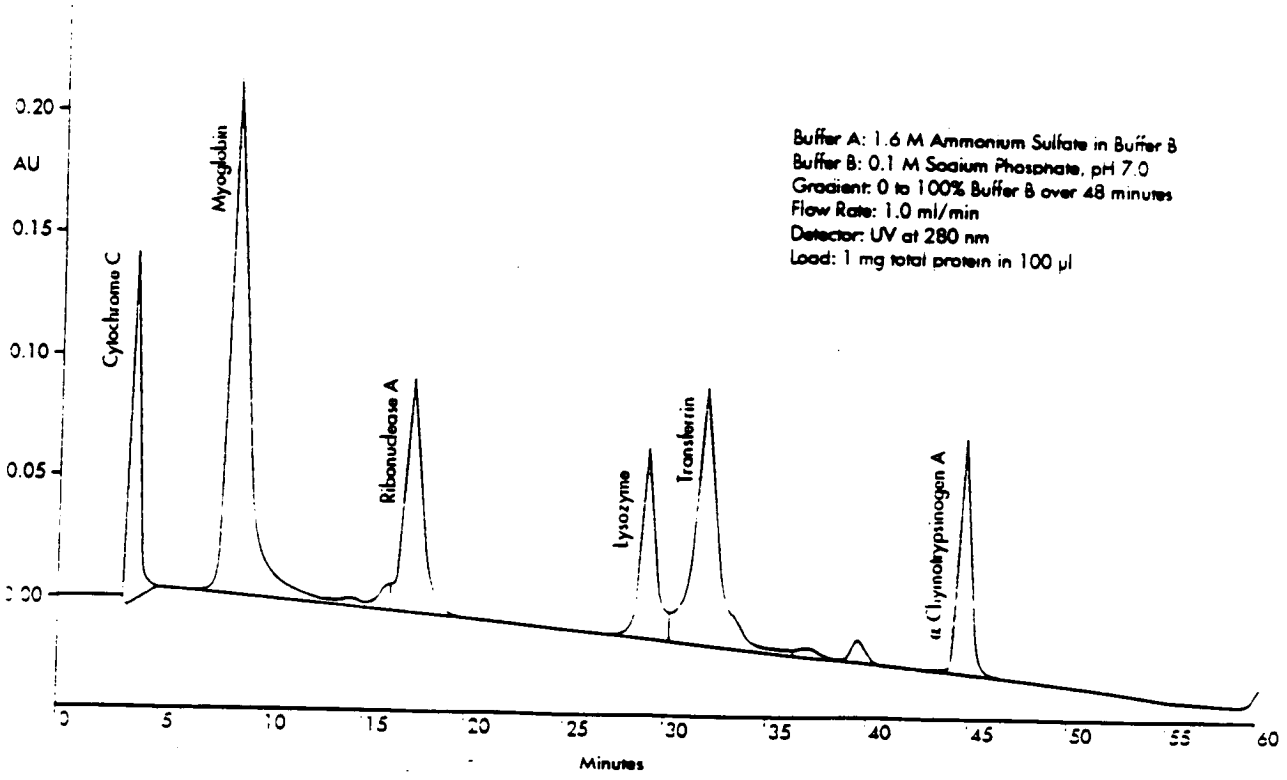
WATERS PH-814 (0.8 x 7.5 cm)



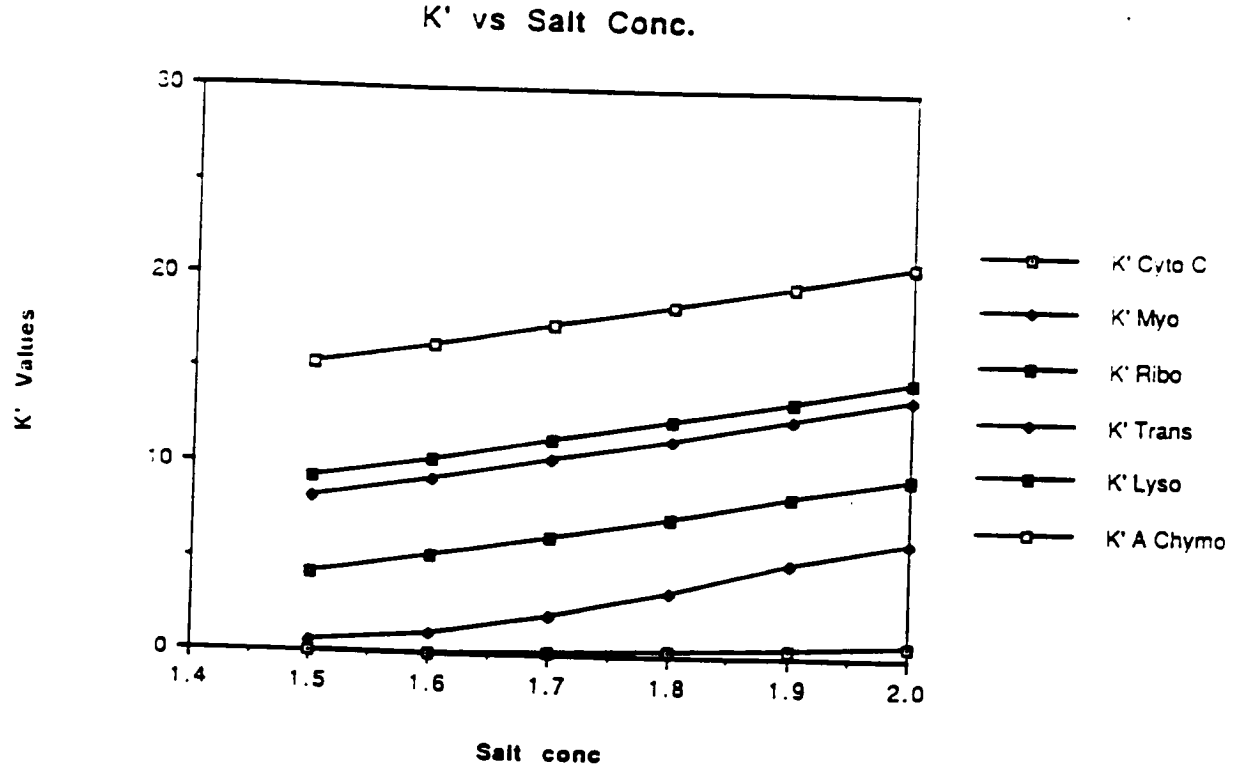


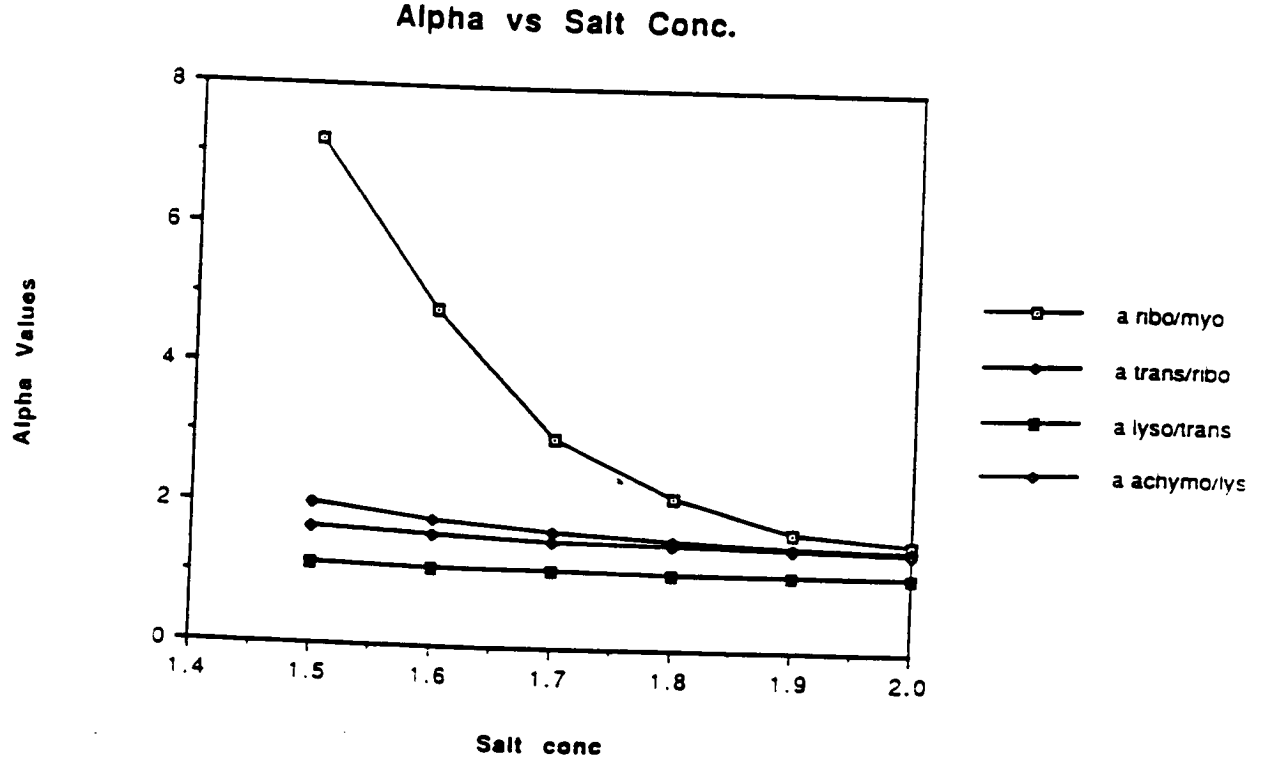
TSK Phenyl 5PW (0.8 X 7.5 cm)





EFFECT OF VARYING THE INITIAL SALT (AMMONIUM SULFATE) MOLARITY (FROM 2.0 TO 1.5M) ON THE K' AND ALPHA VALUES OF THE STANDARD MIXTURE ON THE WATERS PH-814 RESIN





EFFECT OF A DIFFERENT SALT

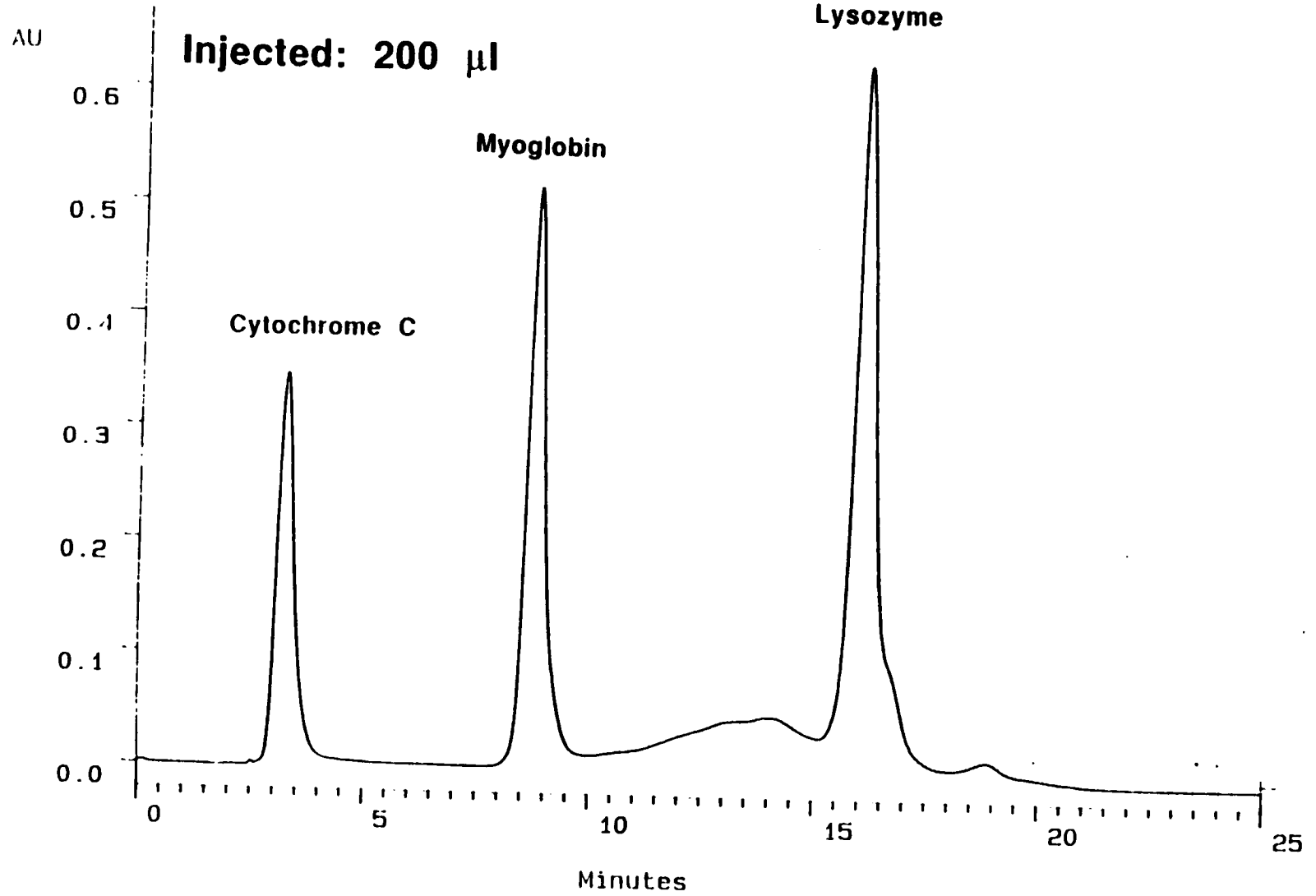
Sample: 0.75 mg/ml of Cytochrome C, Lysozyme, Myoglobin in Buffer A

Gradient: 0 -100% B in 15 minutes at 1.0 ml/minute

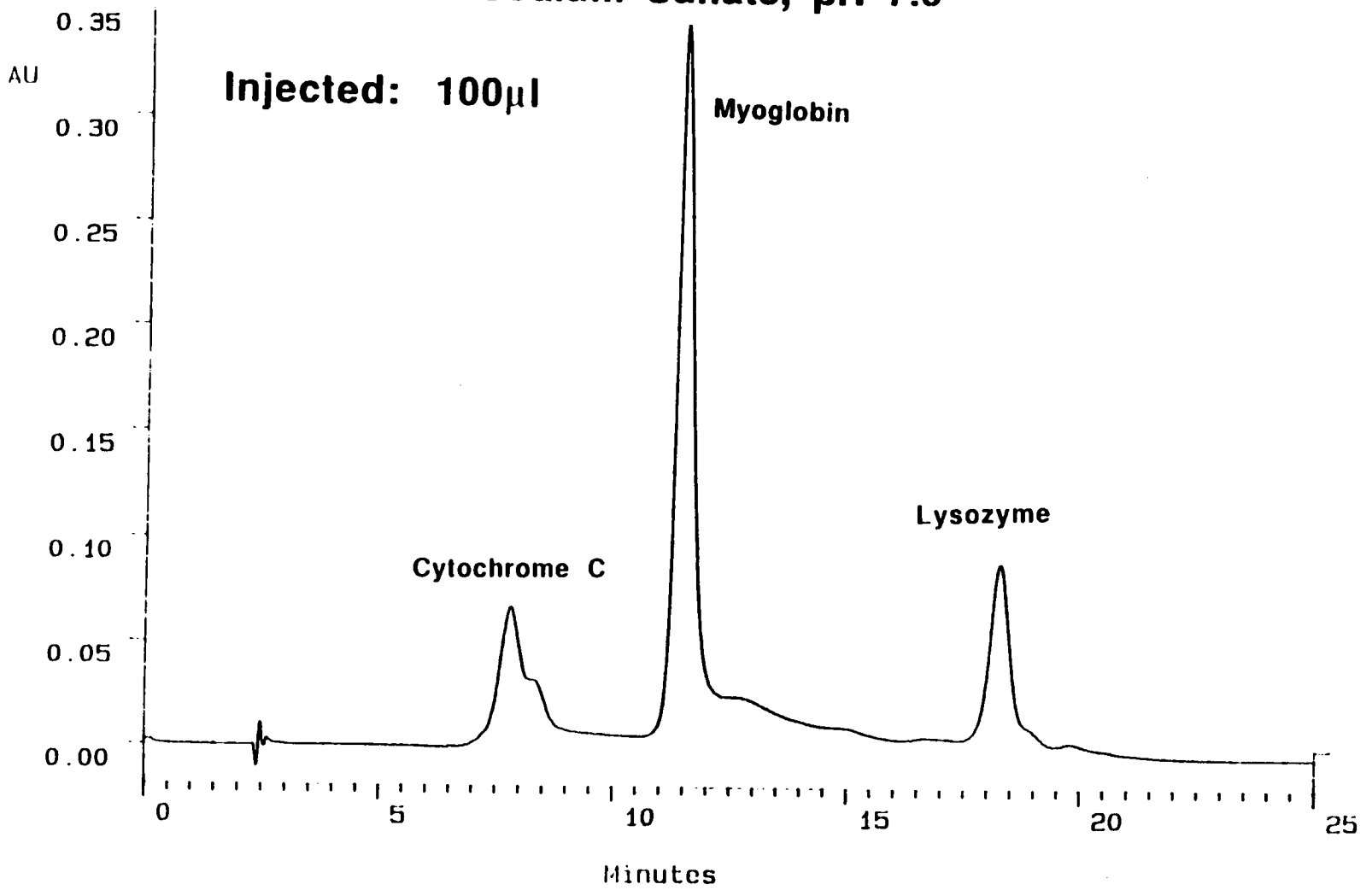
Detector: UV at 280 nm

Buffer A = 1.7M Ammonium Sulfate in Buffer B

Buffer B = 0.1M Sodium Phosphate, pH 7.0



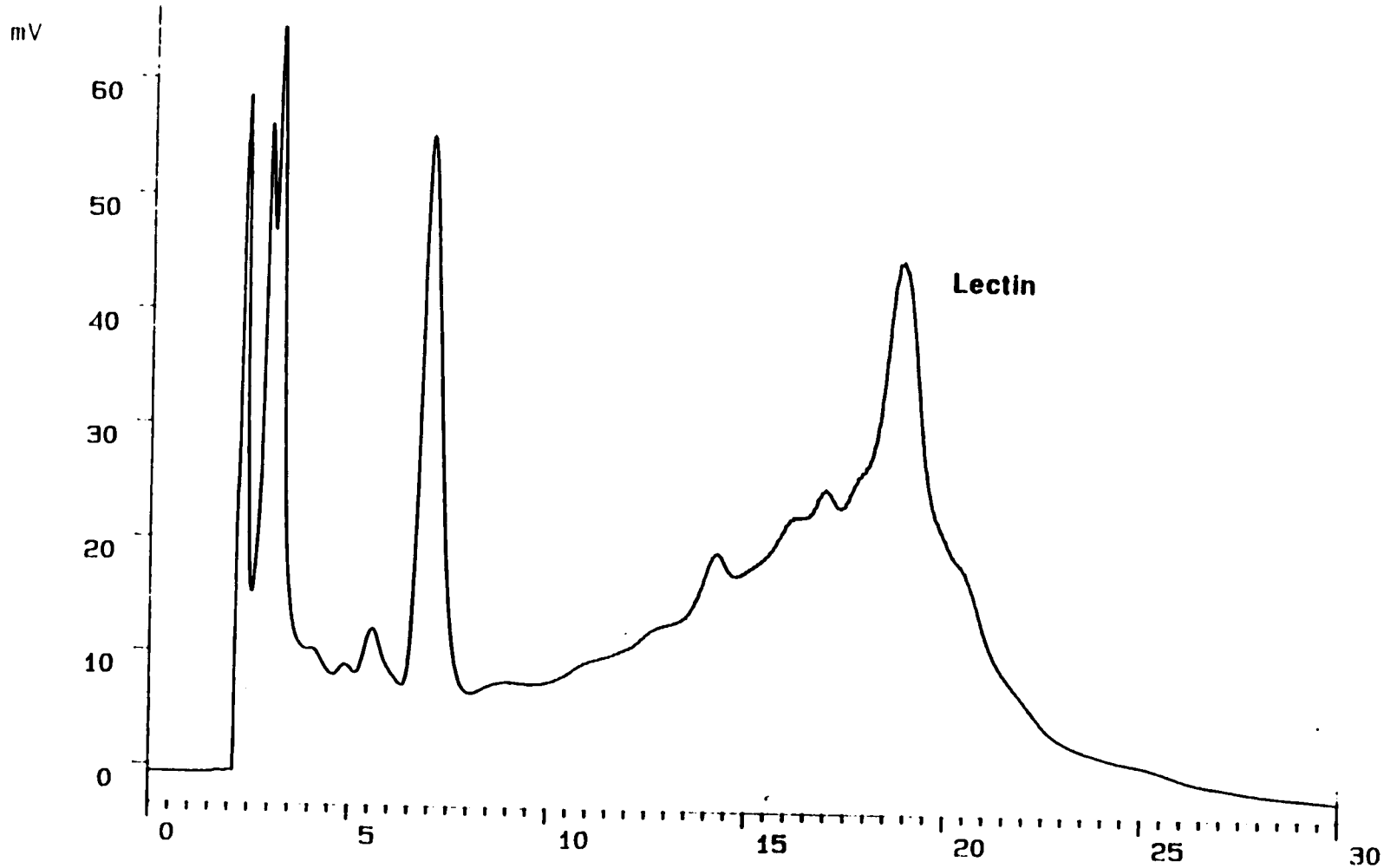
Sample: 0.75 mg/ml of Cytochrome C, Lysozyme, and
1.5mg/ml of myoglobin in Buffer A
Gradient:0 - 100% Buffer B in 15 minutes at 1.0 ml/minute
Detector:UV at 280 nm
Buffer A = 1.7M Sodium Sulfate in Buffer B
Buffer B = 0.1M Sodium Sulfate, pH 7.0



APPLICATIONS

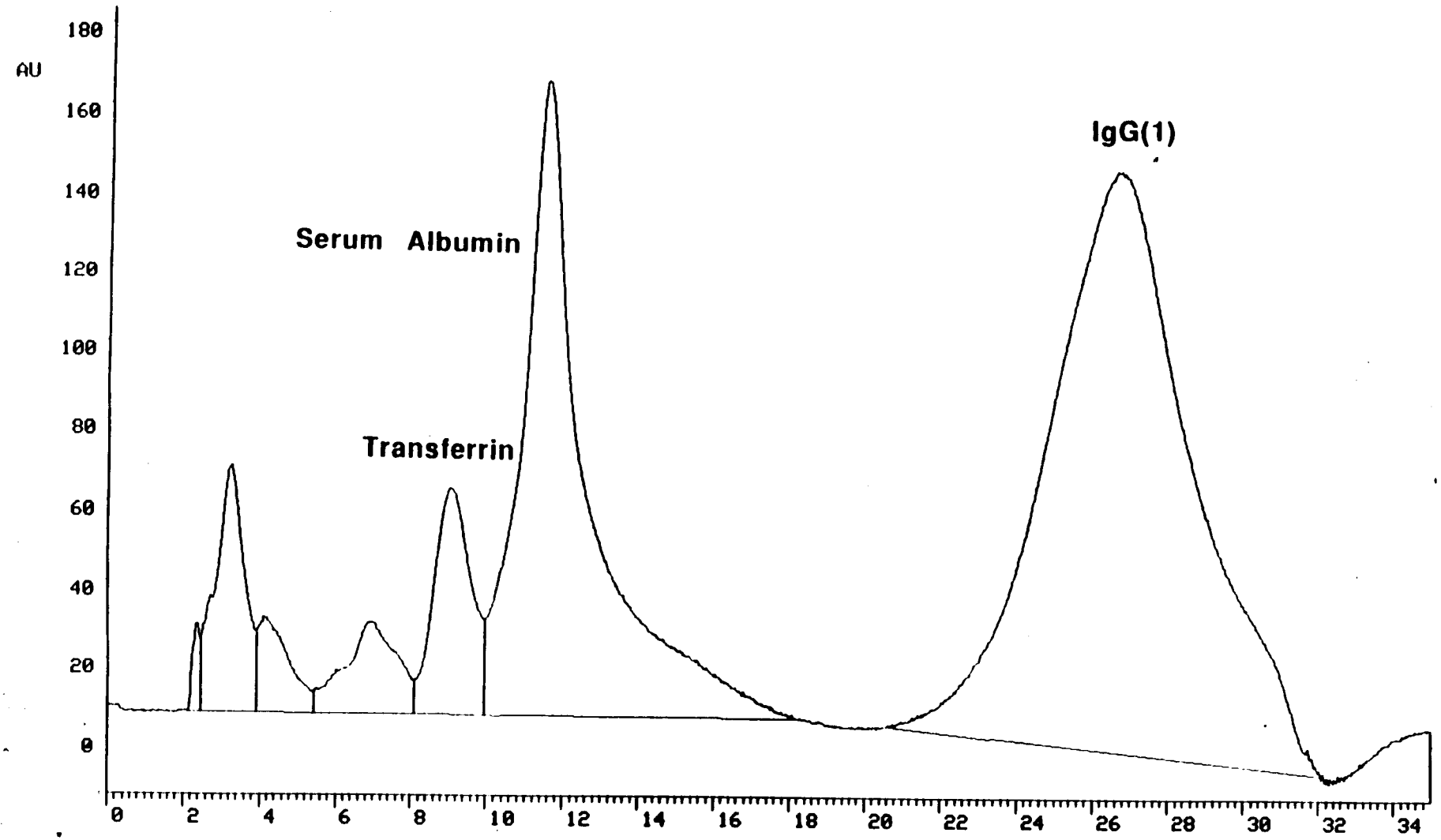
Sample: Wheat Germ Extract

Buffer A = 1.7M Ammonium Sulfate in Buffer B
Buffer B = 0.1M Sodium Phosphate, ph 7.0
Gradient: 0 - 100% B in 15 minutes at 1.0 ml/minute
Detector: UV at 280 nm
Injection: 35 µl



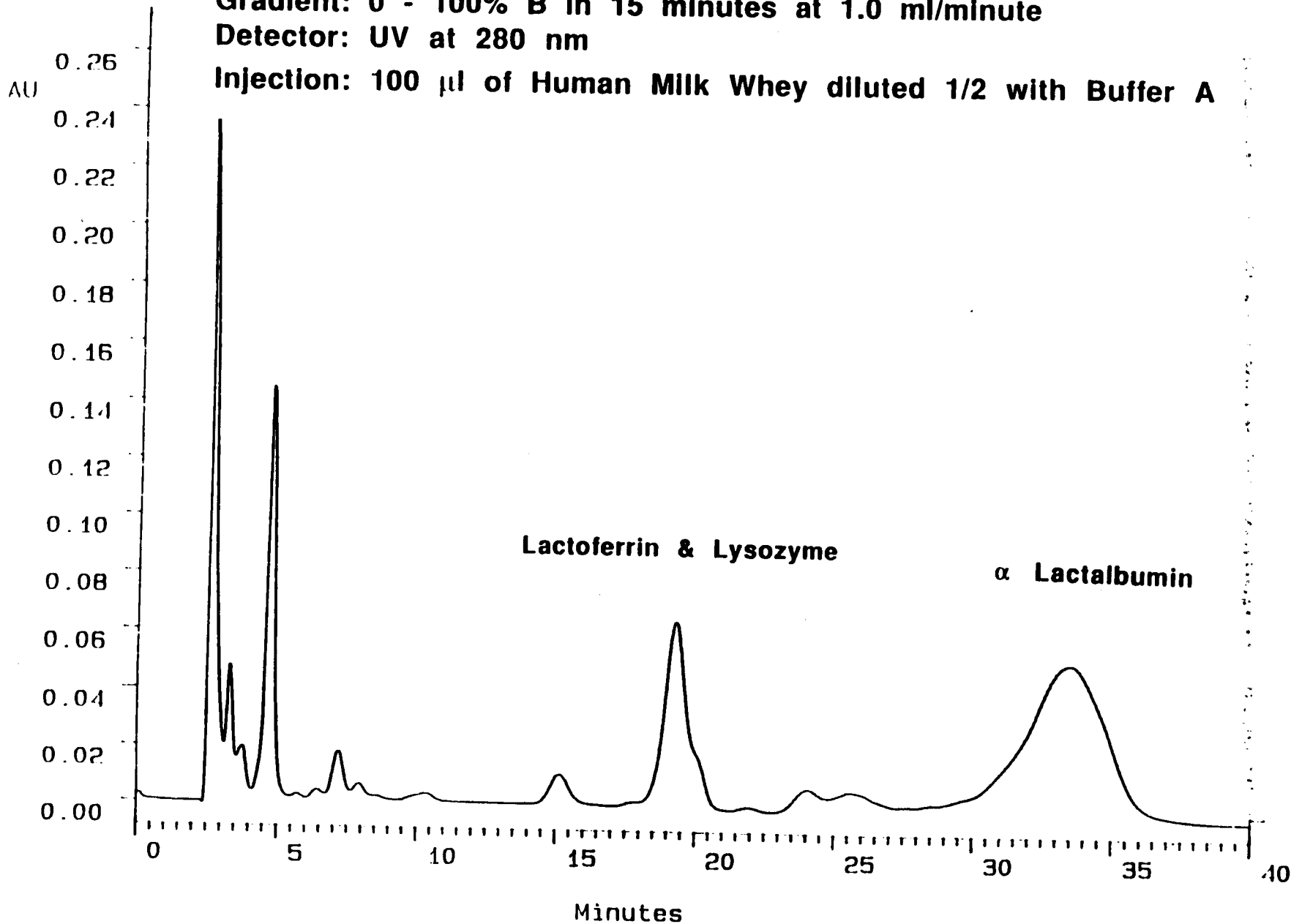
Sample: Mouse Ascites (IgG1)

Buffer A = 1.6M Ammonium Sulfate in Buffer B
Buffer B = 0.1M Sodium Phosphate, pH 7.0
Gradient: Initial: 20% B; Linear Curve to 75% B in 5 minutes, Hold for 5 minutes; Concave gradient to 100 % B in 15 minutes
Detector: UV at 280 nm
Injection: 200 µl of Mouse Acites diluted 1/2 with Buffer B then made 1.4M Ammonium Sulfate with Buffer A



Sample: Human Milk Whey

Buffer A = 1.7M Ammonium Sulfate in Buffer B
Buffer B = 0.1M Sodium Sulfate, pH 7.0
Gradient: 0 - 100% B in 15 minutes at 1.0 ml/minute
Detector: UV at 280 nm
Injection: 100 µl of Human Milk Whey diluted 1/2 with Buffer A



Alpha Values (a = k'2/k'1)

	Waters PH-814		TSK		Pharmacia	
	1.8M	1.6M	1.8M	1.6M	1.8M	1.6M
Ribonuclease A/ Myoglobin	1.83	3.46	1.65	2.78	1.36	2.83
Transferrin/Ribonuclease A	1.57	1.78	1.75	2.1	1.28	1.42
Lysozyme/Transferrin	1.13	1.16	1.09	1.11	0	0
Chymotrypsinogen A/Lysozyme	1.35	1.43	1.49	1.6	1.27	1.3

K' PEAK DATA

k' = (Ve-Vt)/Vt

	PH-814		TSK		Pharmacia	
	1.8M	1.6M	1.8M	1.6M	1.8M	1.6M
Cytochrome C	0.43	0.18	0.17	0	0.31	0.11
Myoglobin	4.14	1.57	3.31	1.36	7.32	2.78
Ribonuclease A	7.57	5.43	5.47	3.78	9.89	7.82
Transferrin	11.92	9.68	9.56 *	7.92 *	12.66**	11.08**
Lysozyme	13.42	11.25	8.75 *	7.11 *	12.66**	11.08**
Chymotrypsinogen A	18.17	16.07	13.06	11.4	16.03	14.45

Ve = Elution Volume

Vt = Total Volume

* = These Proteins Reversed on this Column

** = Not Separated

CONCLUSIONS

Hydrophobic interaction chromatography (HIC) is a good alternative to the more conventional modes of chromatography (such as ion exchange and reversed phase) when measured by its ability to separate proteins of interest from biological matrices.

The results of this study strongly suggest that the addition of the HIC packing to the Protein-Pak™ series increases the number of chromatographic mechanisms by which one can achieve the desired separation without loss of biological activity.

The selectivity of the protein separation was shown to be dependent on the molarity and type of salt used in the starting buffer. Higher molarity salts resulted in greater retention of the proteins.

The Waters PH-814 packing for HIC performed as well as (and in some cases better than) its most well known competitors.